

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Andrew D. Ellington, Jay Hesselberth, Kristin A. Marshall, Michael P. Robertson, Letha Sooter, Eric Davidson, J. Colin Cox, and Timothy Reidel

Serial Number: 09/883,119

Filing Date: June 14, 2001

Group Art Unit: 1653

Examiner: N/A

Application Title: REGULATABLE, CATALYTICALLY ACTIVE NUCLEIC ACIDS

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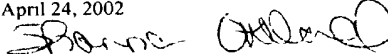
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**BOX: NON-FEE AMENDMENT**  
Commissioner for Patents  
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Prior to examination of the above-identified Application on the merits, please amend the Application as follows:

DETAILED DESCRIPTION OF THE INVENTION

On page 69, delete paragraph [00212] that extends to page 70, of the above-captioned Specification and insert the following:

--[00212] Gp1Wt3.129 (TAA TCT TAC CCC GGA ATT ATA TCC AGC TGC ATG TCA CCA TGC AGA GCA GAC TAT ATC TCC AAC TTG TTA AAG CAA GTT GTC TAT CGT TTC GAG TCA CTT GAC CCT ACT CCC CAA AGG GAT AGT CGT TAG). These oligonucleotides (100 pmol) were annealed and extended with AMV reverse transcriptase (Amersham Pharmacia Biotech, Piscataway, NJ; 45 units) in AMV RT buffer (50 mM Tris-HCl, pH 8.3, 8 mM MgCl<sub>2</sub>, 50 mM NaCl, 1 mM DTT) and dNTPs (200 μM) for 30 minutes at 37° C.

The resulting double-stranded DNA was diluted 1:50 and amplified using primers Spel.24 (TTA TAC TAG TAA TCT ATC TAA ACG (SEQ ID NO:17); 0.4  $\mu$ M) and EcoRI.24 (CCC GGA ATT CTA TCC AGC TGC ATG (SEQ ID NO:18); 0.4  $\mu$ M) in PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.1% Triton X-100, 0.005% gelatin), dNTPs (200  $\mu$ M) and Taq DNA polymerase (Promega, Madison, WI; 1.5 units). The reactions were thermocycled 15 times at 94° C for 30 seconds, 45° C for 30 seconds, 72° C for 1 minute and then purified with a QIAquick PCR purification kit (Qiagen, Valencia, CA).--

On page 70, delete paragraph [00213], of the above-captioned Specification and insert the following:

--[00213] The PCR product was digested with Spel (New England Biolabs, Beverly, MA; 20 units) and EcoRI (50 units) in buffer (50 mM NaCl, 100 mM Tris-HCl, pH 7.5, 10 mM MgCl<sub>2</sub>, 0.025% Triton X-100, 100  $\mu$ g/ml BSA) at 37° C for 60 minutes, purified, and cloned into Spel/EcoRI digested pTZtd1304. The negative control and nucleic acid constructs were made as described except that Gp1Wt3.129 was replaced with oligonucleotides of the appropriate sequence: B11 GCC TGA GTA TAA GGT GAC TTA TAC TTG TAA TCT ATC TAA ACG GGG AAC CTC TCT AGT AGA CAA TCC CGT GCT AAA TGC CTA ACG ACT ATC CCT T (SEQ ID NO:19),--

On page 70, delete paragraph [00214], of the above-captioned Specification and insert the following:

--[00214] Th1P6 GCC TGA GTA TAA GGT GAC TTA TAC TTG TAA TCT ATC TAA ACG GGG AAC CTC TCT AGT AGA CAA TCC CGT GCT AAA TTA TAC CAG CAT CGT CTT GAT GCC CTT GGC AGA TAA ATG CCT AAC GAC TAT CCC TT (SEQ ID NO:20),--

On page 70, delete paragraph [00215], of the above-captioned Specification and insert the following:

--[00215] Th2P6 GCC TGA GTA TAA GGT GAC TTA TAC TTG TAA TCT ATC TAA ACG GGG AAC CTC TCT AGT AGA CAA TCC CGT GCT AAA TTG ATA CCA GCA TCG TCT TGA TGC CCT TGG CAG CAT AAA TGC CTA ACG ACT ATC CCT T (SEQ ID NO:21),--

On page 70, delete, paragraph [00216], of the above-captioned Specification and insert the following:

--[00216] Th3P6 GCC TGA GTA TAA GGT GAC TTA TAC TTG TAA TCT ATC TAA ACG GGG AAC CTC TCT AGT AGA CAA TCC CGT GCA TAC CAG CAT CGT CTT GAT GCC CTT GGC AGG CCT AAC GAC TAT CCC TT (SEQ ID NO:22),--

On page 70, delete paragraph [00217] that extends to page 71, of the above-captioned Specification and insert the following:

--[00217] Th4P6 GCC TGA GTA TAA GGT GAC TTA TAC TTG TAA TCT ATC TAA  
ACG GGG AAC CTC TCT AGT AGA CAA TCC CGT GCT AAA TAT ACC AGC ATC GTC  
TTG ATG CCC TTG GCA GTA AAT GCC TAA CGA CTA TCC CTT (SEQ ID NO:23),--

On page 71, delete paragraph [00218], of the above-captioned Specification and insert the following:

--[00218] Th5P6 GCC TGA GTA TAA GGT GAC TTA TAC TTG TAA TCT ATC TAA  
ACG GGG AAC CTC TCT AGT AGA CAA TCC CGT ATA CCA GCA TCG TCT TGA TGC  
CCT TGG CAG CTA ACG ACT ATC CCT T (SEQ ID NO:24),--

On page 71, delete paragraph [00219], of the above-captioned Specification and insert the following:

--[00219] Th6P6 GCC TGA GTA TAA GGT GAC TTA TAC TTG TAA TCT ATC TAA  
ACG GGG AAC CTC TCT AGT AGA CAA TCC CGT GAT ACC AGC ATC GTC TTG ATG  
CCC TTG GCA GCC TAA CGA CTA TCC CTT (SEQ ID NO:25),--

On page 71, delete paragraph [00220], of the above-captioned Specification and insert the following:

--[00220] Th1P5 TGA GTA TAA GGT GAC TTA TAC TAG TAA TCT ATC TAA ACG  
GGG AAC CTC TAT ACC AGC ATC GTC TTG ATG CCC TTG GCA GAG ACA ATC CCG  
TGC TAA ATT GTA GGA CTG CCC GGG TTC TAC ATA AAT GCC TAA CGA CTA TCC  
CTT (SEQ ID NO:26),--

On page 71, delete paragraph [00221], of the above-captioned Specification and insert the following:

--[00221] Th2P5 TGA GTA TAA GGT GAC TTA TAC TAG TAA TCT ATC TAA ACG  
GGG AAC CTA TAC CAG CAT CGT CTT GAT GCC CTT GGC AGA CAA TCC CGT GCT  
AAA TTG TAG GAC TGC CCG GGT TCT ACA TAA ATG CCT AAC GAC TAT CCC TT  
(SEQ ID NO:27),--

On page 71, delete paragraph [00222], of the above-captioned Specification and insert the following:

--[00222] 3Mex2P6 GTA ATC TAT CTA AAC GGG GAA CCT CTC TAG TAG ACA  
ATC CCG TGC TAA ATT GAT ACC AGC ATC GTC TTG ATG CCA TTG GCA GCA TAA  
ATG CCT AAC GAC TAT CCC TT (SEQ ID NO:28),--



## IN THE DRAWINGS

REMARKS

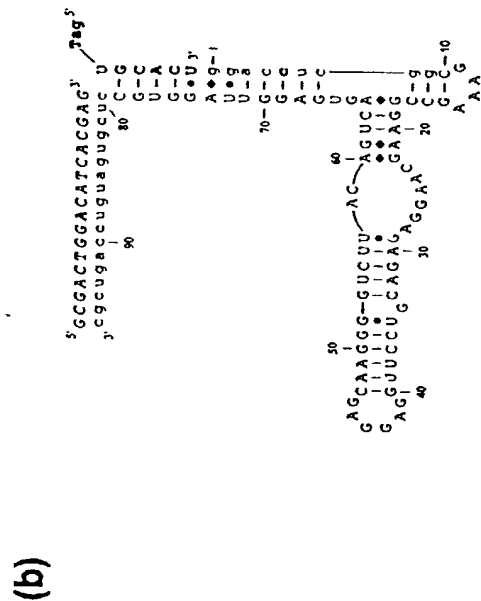
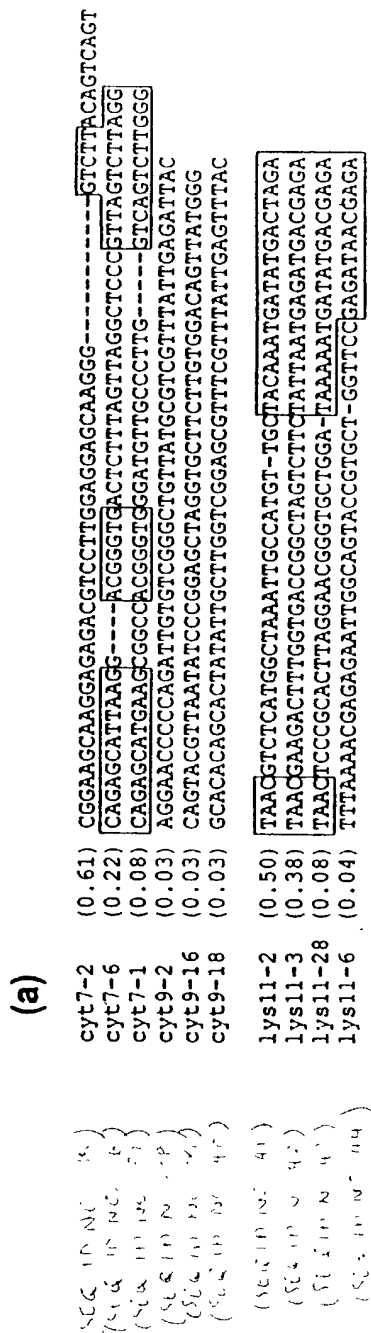
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Figure 19



In re Application of: Andrew D. Ellington, Jay Hesselberth, Kristin A. Marshall, Michael P. Robertson, Letha Sooter, Eric Davidson, J. Colin Cox, and Timothy Reidel

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**MARKED-UP COPY OF AMENDED PARAGRAPHS FOR  
PRELIMINARY AMENDMENT IN ACCORDANCE WITH C.F.R. § 1.121**

[AMENDED] [00212] Gp1Wt3.129 (TAA TCT TAC CCC GGA ATT ATA TCC AGC TGC ATG TCA CCA TGC AGA GCA GAC TAT ATC TCC AAC TTG TTA AAG CAA GTT GTC TAT CGT TTC GAG TCA CTT GAC CCT ACT CCC CAA AGG GAT AGT CGT TAG) [(SEQ ID NO:17)]. These oligonucleotides (100 pmol) were annealed and extended with AMV reverse transcriptase (Amersham Pharmacia Biotech, Piscataway, NJ; 45 units) in AMV RT buffer (50 mM Tris-HCl, pH 8.3, 8 mM MgCl<sub>2</sub>, 50 mM NaCl, 1 mM DTT) and dNTPs (200 μM) for 30 minutes at 37° C. The resulting double-stranded DNA was diluted 1:50 and amplified using primers SpeI.24 (TTA TAC TAG TAA TCT ATC TAA ACG (SEQ ID NO:[18]17); 0.4 μM) and EcoRI.24 (CCC GGA ATT CTA TCC AGC TGC ATG (SEQ ID NO:[19]18); 0.4 μM) in PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.1% Triton X-100, 0.005% gelatin), dNTPs (200 μM) and Taq DNA polymerase (Promega, Madison, WI; 1.5 units). The reactions were thermocycled 15 times at 94° C for 30 seconds, 45° C for 30 seconds, 72° C for 1 minute and then purified with a QIAquick PCR purification kit (Qiagen, Valencia, CA).

[AMENDED] [00213] The PCR product was digested with SpeI (New England Biolabs, Beverly, MA; 20 units) and EcoRI (50 units) in buffer (50 mM NaCl, 100 mM Tris-HCl, pH 7.5, 10 mM MgCl<sub>2</sub>, 0.025% Triton X-100, 100 μg/ml BSA) at 37° C for 60 minutes, purified, and cloned into SpeI/EcoRI digested pTZtd1304. The negative control and nucleic acid constructs were made as described except that Gp1Wt3.129 was replaced with oligonucleotides of the appropriate sequence: B11 GCC TGA GTA TAA GGT GAC TTA TAC TTG TAA TCT ATC TAA ACG GGG AAC CTC TCT AGT AGA CAA TCC CGT GCT AAA TGC CTA ACG ACT ATC CCT T (SEQ ID NO:[20]19),

[AMENDED] [00214] Th1P6 GCC TGA GTA TAA GGT GAC TTA TAC TTG TAA TCT ATC TAA ACG GGG AAC CTC TCT AGT AGA CAA TCC CGT GCT AAA TTA TAC CAG CAT CGT CTT GAT GCC CTT GGC AGA TAA ATG CCT AAC GAC TAT CCC TT (SEQ ID NO:[21]20),

[AMENDED] [00215] Th2P6 GCC TGA GTA TAA GGT GAC TTA TAC TTG TAA TCT ATC TAA ACG GGG AAC CTC TCT AGT AGA CAA TCC CGT GCT AAA TTG ATA

CCA GCA TCG TCT TGA TGC CCT TGG CAG CAT AAA TGC CTA ACG ACT ATC CCT  
T (SEQ ID NO:[22]21),

[AMENDED] [00216] Th3P6 GCC TGA GTA TAA GGT GAC TTA TAC TTG TAA  
TCT ATC TAA ACG GGG AAC CTC TCT AGT AGA CAA TCC CGT GCA TAC CAG CAT  
CGT CTT GAT GCC CTT GGC AGG CCT AAC GAC TAT CCC TT (SEQ ID NO:[23]22),--

[AMENDED] [00217] Th4P6 GCC TGA GTA TAA GGT GAC TTA TAC TTG TAA  
TCT ATC TAA ACG GGG AAC CTC TCT AGT AGA CAA TCC CGT GCT AAA TAT ACC  
AGC ATC GTC TTG ATG CCC TTG GCA GTA AAT GCC TAA CGA CTA TCC CTT (SEQ  
ID NO:[24]23),

[AMENDED] [00218] Th5P6 GCC TGA GTA TAA GGT GAC TTA TAC TTG TAA  
TCT ATC TAA ACG GGG AAC CTC TCT AGT AGA CAA TCC CGT ATA CCA GCA TCG  
TCT TGA TGC CCT TGG CAG CTA ACG ACT ATC CCT T (SEQ ID NO:[26]24),

[AMENDED] [00219] Th6P6 GCC TGA GTA TAA GGT GAC TTA TAC TTG TAA  
TCT ATC TAA ACG GGG AAC CTC TCT AGT AGA CAA TCC CGT GAT ACC AGC ATC  
GTC TTG ATG CCC TTG GCA GCC TAA CGA CTA TCC CTT (SEQ ID NO:[27]25),

[AMENDED] [00220] Th1P5 TGA GTA TAA GGT GAC TTA TAC TAG TAA TCT  
ATC TAA ACG GGG AAC CTC TAT ACC AGC ATC GTC TTG ATG CCC TTG GCA GAG  
ACA ATC CCG TGC TAA ATT GTA GGA CTG CCC GGG TTC TAC ATA AAT GCC TAA  
CGA CTA TCC CTT (SEQ ID NO:[28]26),

[AMENDED] [00221] Th2P5 TGA GTA TAA GGT GAC TTA TAC TAG TAA TCT  
ATC TAA ACG GGG AAC CTA TAC CAG CAT CGT CTT GAT GCC CTT GGC AGA CAA  
TCC CGT GCT AAA TTG TAG GAC TGC CCG GGT TCT ACA TAA ATG CCT AAC GAC  
TAT CCC TT (SEQ ID NO:[29]27),

[AMENDED] [00222] 3Mex2P6 GTA ATC TAT CTA AAC GGG GAA CCT CTC TAG  
TAG ACA ATC CCG TGC TAA ATT GAT ACC AGC ATC GTC TTG ATG CCA TTG GCA  
GCA TAA ATG CCT AAC GAC TAT CCC TT (SEQ ID NO:[30]28),

[AMENDED] [00223] Th2P6.D GTA ATC TAT CTA AAC GGG GAA CCT CTC TAG  
TAG ACA ATC CCG TGC TAA ATT GAT ACC AGC ATC GTC TTG ATG CCC TTG GTT  
GCA TAA ATG CCT AAC GAC TAT CCC TT (SEQ ID NO:[31]29),

[AMENDED] [00224] FMN1P6 GCC TGA GTA TAA GGT GAC TTA TAC TTG TAA  
TCT ATC TAA ACG GGG AAC CTC TCT AGT AGA CAA TCC CGT GCT AAA TTA GGA  
TAT GCT TCG GCA GAA GGA TAA ATG CCT AAC GAC TAT CCC TT (SEQ ID  
NO:[32]30), and

[AMENDED] [00225] FMN2P6 GCC TGA GTA TAA GGT GAC TTA TAC TTG TAA  
TCT ATC TAA ACG GGG AAC CTC TCT AGT AGA CAA TCC CGT GCT AAA TTG AGG  
ATA TGC TTC GGC AGA AGG CAT AAA TGC CTA ACG ACT ATC CCT T (SEQ ID  
NO:[33]31).



[AMENDED] [00226] In vitro transcription. The introns were PCR-amplified with 5' le (GAT AAT ACG ACT CAC TAT AAT GGC ATT ACC GCC TTG T) (SEQ ID NO:[34]32) and GM24 (GCT CTA GAC TTA GCT ACA ATA TGA AC) (SEQ ID NO:[35]33) in 25  $\mu$ l reactions under the conditions stated above and cycled 20 times. A portion of the reaction (5  $\mu$ l) was run on a 3% agarose gel and the PCR product band was stabbed with a pipette tip. The agarose plug was added to a fresh PCR reaction (100  $\mu$ l) and cycled 15 times; DNA was purified using a QIAquick kit and quantitated. The PCR product (2  $\mu$ g in 50  $\mu$ l) was added to an *in vitro* transcription reaction containing Ampliscribe T7 RNA polymerase (Epicentre), RNase inhibitor (GIBCO BRL, Rockville, MD; 5 units), low Mg<sup>2+</sup> buffer (30 mM Tris-HCl, pH 8, 7.5 mM DTT, 4.5 mM MgCl<sub>2</sub>, 1.5 mM spermidine), UTP (1.25 mM), ATP (2.5 mM), GTP (2.5 mM), CTP (7.5 mM) and aP32-labeled UTP (NEN, Boston, MA; 20  $\mu$ Ci; 3000 mCi/mmol), and incubated at 37° C for 2 hours. DNase (GIBCO BRL, 295 units) was added and the reaction was incubated at 37° C for an additional 30 minutes. The RNA was purified using Centri-Sep columns (Princeton Separations, Adelphia, NJ) and quantitated.

[AMENDED] [00248] Aptazyme Array and Titration of Individual Aptazymes. Arrayed aptazyme assay were carried out by first annealing 100 pmol of ribozyme with 120 pmol of 18.90A (5' GCGACTGGACATCACGAG 3')[(SEQ ID NO:36)]. Following addition of buffer (30 mM Tris-HCl, pH 7.5, 50 mM NaCl, 60 mM MgCl<sub>2</sub>), 120 pmol of substrate (S28A-biotin, 5' biotin-AAAAAAAAAAAAAAAAAAAAAugcacu 3', (SEQ ID NO:[37]34) ribonucleotides in lowercase) was added. The reaction mixture was scaled to accommodate multiple aliquots for each corresponding well of the array. After aliquotting 50  $\mu$ l into each well of an 96-well PCR plate (MJ Research), 50  $\mu$ l of ligand in buffer was added. Ligand concentrations for Fig. 29 were: 1  $\mu$ M 18.90A, 0.5 mM flavin mononucleotide (FMN), 5  $\mu$ M lysozyme, 1  $\mu$ M Rev peptide, 1 mM ATP, and 1 mM theophylline.